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VARIATIONS IN THE TIME COURSE OF THE SYNCHRONIZATION OF INTERCOSTAL MOTONEURONES IN THE CAT

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SUMMARY

1. Synchronization of intercostal motoneurones was studied by the construction of cross-correlation histograms which related the firing times of paired groups of efferent inspiratory or expiratory discharges recorded from filaments of the external or internal nerves of anaesthetized or decerebrate cats.

2. The principal feature of the histograms was always a central peak but the time course of the central peak showed considerable variation. Three forms of synchronization were defined on the basis of the time course of the central peak: (i) short-term synchronization (Sears & Stagg, 1976), where the peak was narrow, extending over about ± 3 ms but sometimes with weak shoulders to about ± 5 ms; (ii) broad-peak synchronization where the peak was wider than this (often ± 20 ms or more) but where there were no strong periodicities; (iii) high-frequency oscillation (h.f.o.) synchronization, which was named from the related phenomena in medullary and phrenic recordings (Cohen, 1979), where there were periodic peaks on either side of the central peak with a frequency in the range 60–120 Hz. Combinations of these forms of synchronization were seen in some histograms.

3. When different animals were compared, broad peak synchronization was seen in association with light anaesthesia and with polysynaptic excitation of the motoneurones from muscle spindle afferents.

4. In individual animals, additional anaesthesia depressed both broad peak and h.f.o. synchronization.

5. Raising P_{A,CO_2}, which increased the respiratory drive to the motoneurones, favoured short-term or h.f.o. synchronization at the expense of broad-peak synchronization.

6. In three decerebrate animals only short-term or h.f.o. synchronization was seen.

7. Spinal cord lesions above or below the segments of interest promoted broad-peak synchronization, even with high P_{A,CO_2} or deep anaesthesia.

8. We conclude: (i) that short-term synchronization, due mainly to the branching of presynaptic axons, is generated mainly by those axons which transmit the respiratory drive, that drive providing most of the excitation of the motoneurones

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in moderately deep anaesthesia; (ii) that h.f.o. synchronization arises from the periodic synchronization of the discharges in these same presynaptic axons; (iii) that broad-peak synchronization is generated by the activity of other presynaptic neurones whose discharges are also synchronized, but aperiodically, these neurones most likely including spinal cord interneurones which are active in light anaesthesia or when released by spinal cord lesions.

9. These conclusions are supported by comparisons between intracellular recordings from inspiratory motoneurones in animals showing different forms of motoneurone synchronization, the comparison including the measurements of 'average common excitation' (a.c.e.) potentials (Kirkwood & Sears, 1978).

INTRODUCTION

Spinal motoneurones form the final common path of Sherrington (1906). They can be brought to firing by several different input systems each employing perhaps only a fraction of the total estimated number of excitatory synapses. At the same time each of the input fibres may diverge to excite a large proportion of the motoneurones innervating a given muscle, up to perhaps 100% (e.g. Mendell & Henneman, 1968).

For external intercostal motoneurones of the same or adjacent spinal cord segments in the anaesthetized cat, Sears & Stagg (1976) demonstrated that one consequence of such divergence was a short-term synchronization of motoneurone firing times, reflected in a narrow central peak (± 3 ms) in the cross-correlation histogram between paired groups of motoneurone discharges. Sears & Stagg (1976) pointed out that such synchronization was an inevitable result of the known divergence of individual presynaptic axons to the different motoneurones and would be expected to occur independently of any synchronization that might be present between the discharges of individual presynaptic axons. They interpreted their observations in terms of the branched axon hypothesis alone. This interpretation was supported by later experiments of Kirkwood & Sears (1978), who measured the intracellular analogue of the synchronization, the 'average common excitation' (a.c.e.) potential. They showed via a quantitative model that the time course of this potential and of the short-term synchronization from Sears & Stagg (1976) were jointly explained by the original hypothesis, any contribution from presynaptic synchronization being likely to be small. Furthermore, the model predicted the relationship between a unitary e.p.s.p. and the raised probability of firing that it creates in a motoneurone. This prediction has recently been verified (Kirkwood & Sears, 1982*a*, *b*) thus confirming the original interpretation.

It has also been shown that in man the motoneurones supplying the intrinsic hand muscles (Dietz, Bischofberger, Wita & Freund, 1976; Datta & Stephens, 1980) as well as those supplying the internal intercostal muscle or the triceps surae muscles (Sears & Stagg, 1976; Dietz *et al.* 1976) are similarly synchronized, although in these cases the durations of the peaks in the cross-correlation histograms are not always as narrow as those for the external intercostal motoneurones of the anaesthetized cat. The origin of these forms of synchronization cannot therefore be confidently ascribed to the branched presynaptic axon mechanism alone.

The animals used by Sears & Stagg (1976) and Kirkwood & Sears (1978) were

moderately deeply anaesthetized with sodium pentobarbitone. Here we describe additional experiments of the same kind, but where the state of the animal, in particular its anaesthetic level, varied. We found that synchronization of intercostal motoneurones can have a variety of time courses and we give evidence that distinction may be made between the different synaptic inputs responsible for the generation of these different patterns. Such evidence should be of particular use in interpreting the measurements in man where direct access to motoneurone inputs is difficult. The distribution of the effects between different intercostal segments, which gives further information about the functional distribution of the inputs responsible, is described in the following paper (Kirkwood, Sears, Stagg & Westgaard, 1982). The results of the present paper also serve as control measurements for a later paper which describes the use of similar methods to study the chronic effects of partial central deafferentation of motoneurones (Kirkwood, Sears & Westgaard, 1981*a*, and in preparation).

Some of the experiments have been briefly reported in preliminary communications (Kirkwood, Sears & Stagg, 1974; Kirkwood, Sears & Westgaard, 1981*c*).

METHODS

The data for this paper come largely from experiments already described (Sears & Stagg, 1976; Bainton, Kirkwood & Sears, 1978; Kirkwood & Sears, 1978; 1982*a*, *b*; Kirkwood, Sears & Westgaard, 1981*b*), although another seven cats were prepared specially for the experiments described here. Briefly, in all these experiments branches of the external intercostal nerves (filaments) in segments T_2 to T_{10} of cats anaesthetized with sodium pentobarbitone were prepared for the recording of inspiratory efferent discharges. In a few experiments similar recordings were made from the expiratory discharges of internal intercostal nerve filaments. Most cats were paralysed with gallamine triethiodide and artificially respired with various O_2/CO_2 or air/CO_2 mixtures. Some experiments required the P_{A,CO_2}, which was measured as the end-tidal CO_2 concentration, to be varied. This was achieved by adjusting the stroke volume of the respirator (set at high rate, 52/min) to the volume which gave the required lowest value of P_{A,CO_2} when the animal was ventilated with air or O_2 alone, and then adding CO_2 to the respirator input as required, with the stroke volume maintained constant. Depending on the level of anaesthesia, the eupnoeic level of end-tidal CO_2 was 4–5 % before the animal was paralysed. Most often, the CO_2 added after paralysis was adjusted to give a slightly raised end-tidal level (5–7 %) so as to ensure suitably strong inspiratory discharges in the more caudal segments (T8 or T9) for the experiments on the spatial distribution of synchronization (Kirkwood *et al.* 1982).

Some experiments required measurements to be made at different levels of anaesthesia. This was achieved by making measurements before and after a supplementary dose of pentobarbitone. In some instances a change in anaesthetic level was inferred *post hoc* from the experimental protocols of the times of the anaesthetic supplements. In other instances the aim of the experiment was to maintain the animal specifically lightly anaesthetized and then to give a supplement either of the same size as our usual supplement (3 mg/kg) or larger. A lightly anaesthetized animal was one where there was a moderate but unsustained withdrawal of the forelimb to a strong pinch, but no reflex in the hind limb to a similar stimulus (Sears, 1964*a*; cf. Petersén, 1952). The blood pressure (measured via a femoral arterial cannula) and respiration showed no change to a very strong pinch to the forepaw. The respiratory rate even under Flaxedil was relatively fast (about 20/min) and might be slightly irregular. A deeply anaesthetized animal showed no somatic reflexes and a slower respiratory rate, sometimes as low as 6/min (cf. Fig. 9). In paralysed animals anaesthesia was judged either by reflexes at intervals, when paralysis had worn off, or by the pattern of respiration and blood pressure and their responses to noxious stimuli during paralysis.

Filaments were prepared in several segments and recordings were made from up to seven filaments simultaneously. Data were analysed by measuring cross-correlation histograms (pre- and post-stimulus time histograms) according to the methods of Sears & Stagg (1976) from the multi-unit

discharges of α-motor axons in each filament. In the analyses of this paper all the α-discharges of one filament were taken as a single population of spikes. α- and γ-spikes were separated on the basis of spike height according to the criteria of Sears (1964*a*). In some of the later experiments, data were analysed to give the same type of cross-correlation histograms but by the use of a different computer, a PDP 11/34, and programmes written by Mr J. Davies. Except where noted, the bin width of the histograms was 1 ms.

In some of the experiments intracellular recordings were made from external intercostal motoneurones, as described in Kirkwood & Sears (1978, 1981*a*) In these experiments the intracellular analogue of the cross-correlation histogram, the 'average common excitation' (a.c.e.) potential (Kirkwood & Sears, 1978) was measured by averaging the intracellularly recorded synaptic noise with an averager (DL 4000, Data, Labs, Ltd) triggered by the efferent inspiratory discharges. Some of these averages were also carried out on the PDP 11/34 computer by means of further programmes written by Mr J. Davies.

In some animals lesions to the spinal cord (transections or partial transections) were made. These were carried out under visual guidance using a piece of razor blade or a no. 11 scalpel blade. At least half an hour (usually more) was allowed after making the lesions before taking records for analysis so that the effects of injury discharges should be minimized. Overt signs of excitation, as reflected in the filament discharges, died away within a minute or two of making the cut. Full transections were confirmed visually. Partial transections were mapped histologically post mortem.

Two cats were decerebrated mid-collicularly under anaesthesia (thiopentone sodium 40 mg/kg) and one was similarly prepared under halothane anaesthesia. Anaesthesia was subsequently discontinued for all three preparations.

RESULTS

Different types of synchronization

A cross-correlation histogram from a cat moderately deeply anaesthetized with sodium pentobarbitone is shown in Fig. 1*A* and *B*. The same histogram is shown at two different time scales but with the same bin width in order to emphasize the distinction between the gently curving base line (*A*), representing the synchronization of these motoneurones over the time scale of the respiratory cycle, and the narrow central peak (*B*). Following Sears & Stagg (1976, see their Fig. 6), we regard the curved base line as a non-stationarity in the data quite separate from the stochastic types of synchronization that are superimposed upon it and which are the subject of this paper. For this histogram, the animal was in an anaesthetic state similar to that used by Sears & Stagg (1976). Correspondingly the narrow peak in *A* and *B* is exactly of the form they recorded.

However, in a more recent series of experiments to measure spike-triggered average e.p.s.p.s from spindle afferents, some of the animals were more lightly anaesthetized, and in some of these animals there was clear evidence of polysynaptic excitation of motoneurones from the individual spindle afferents in addition to the monosynaptic excitation observable in all the animals. We took this polysynaptic excitation as direct evidence for a raised level of spinal cord interneuronal activity and we therefore considered it worthwhile looking for signs of this activity in the motoneurone cross-correlation histograms.

Fig. 1*C* and *D* shows a histogram from one of these animals. Like Fig. 1*B*, the histogram in *D* has a narrow central peak, but in addition there is a much wider component extending to around ± 20 ms. This component is clearly separable from the respiratory non-stationarity (Fig. 1*C*). There is not a sharp division in the histogram of Fig. 1*D* between the narrow peak and the broader one, although a

sharper inflexion was sometimes present. Nevertheless, because many animals gave no trace of such a broad base to the central peak we are confident that this type of peak is a separate category of synchronization and will therefore refer to it as broad-peak synchronization.

The animal used for the analysis of Fig. 1*C* and *D* was the one which gave the clearest examples of polysynaptic spike-triggered average e.p.s.p.s (Fig. 11*D* and *E*

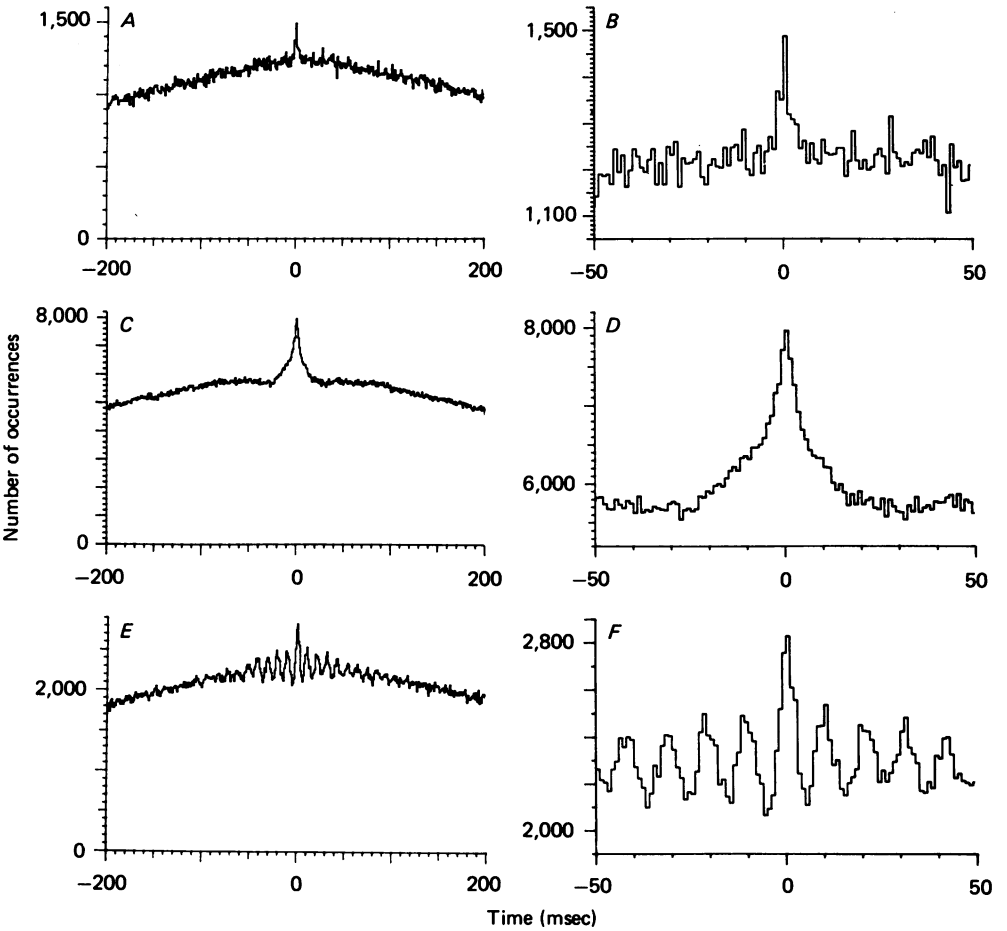

Fig. 1. Cross-correlation histograms showing three different forms of motoneurone synchronization, in each case between the efferent discharges of T6 and T7. Each histogram in left hand column, *A*, *C*, *E*, from a different animal: *A*, moderately deeply anaesthetized, end-tidal CO_2 5·5 %; *C*, *E* lightly anaesthetized, end-tidal CO_2 4·0 and 6·0 % respectively. *B*, *D*, *F*, the same histograms with different time and amplitude scales.

in Kirkwood & Sears, 1982*a*), whereas the animal used in Fig. 1*A* and *B* was one in which there were no signs of polysynaptic components in the e.p.s.p.s (Fig. 11*A–C* in Kirkwood & Sears, 1982*a*). The other two animals which gave polysynaptic e.p.s.p.s in that study also showed broad-peak synchronization between their motoneurone discharges.

In three other lightly anaesthetized animals an additional component was present in the cross-correlation histogram. This component consisted of a highly periodic wave form with a frequency of 60–120 Hz (95·2 Hz in Fig. 1*E* and *F*). The obvious explanation for this is that the behaviour of the intercostal motoneurones is linked to the synchronization of respiratory neurones in the medulla and of phrenic

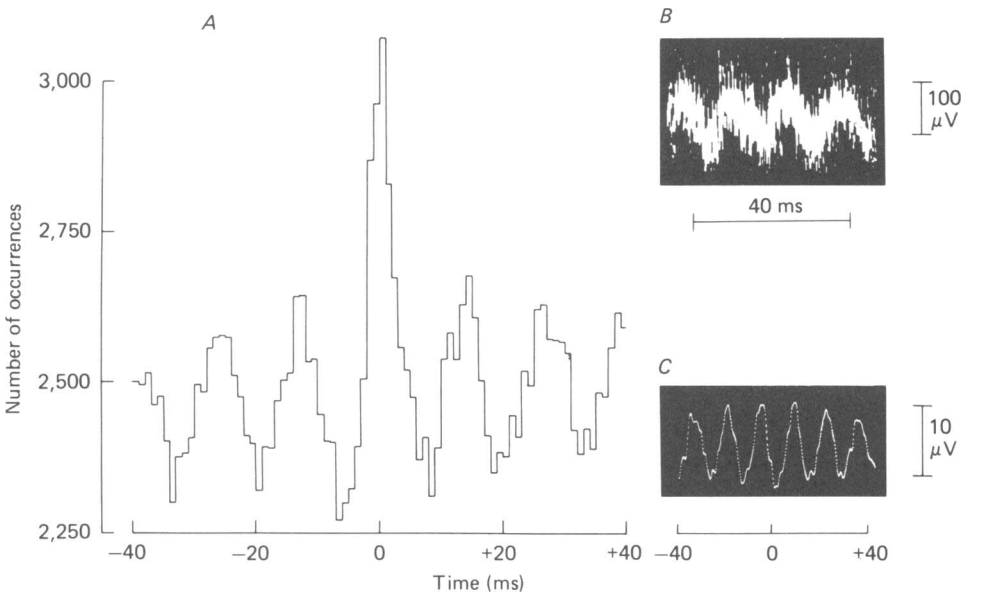

Fig. 2. Identification of h.f.o. synchronization in a decerebrate cat. *A*, cross-correlation histogram between the α-efferent discharges of T6 and T7. *B*, extracellular recording during inspiration from an expiratory recording site in the medulla (2·5 mm caudal and 3·5 mm lateral to obex); superimposed sweeps triggered by positive going deflexions. *C*, average of medullary signal triggered by α-efferent spikes in T7 (589 sweeps).

motoneurones, which has often been observed in this frequency range (see Cohen, 1979 for review). This explanation is strengthened by observations in one animal decerebrated under thiopentone sodium anaesthesia 15 h previously (Fig. 2). In this animal recording was made from an extracellular micro-electrode situated at an expiratory site in the caudal part of nucelus retroamibigualis in the medulla. During inspiration, when no clear spike activity was observable, oscillatory potentials up to 100 μV in amplitude were present with a frequency of 75 Hz (Fig. 2*B*). The cross-correlation histogram between the inspiratory discharges of filaments in T6 and T7 showed the same periodicity (13 ms) (Fig. 2*A*). When an averager was triggered by α-motoneurone spikes from the T7 filament, the average of the medullary recording also showed a relatively undamped periodic wave form at 75 Hz (Fig. 2*C*). The amplitude was only 10 μV, as compared to the maximum value of about 100 μV for the original recording, but because the oscillatory component in the cross-correlation histogram (ignoring the strong central peak) represents only about 10% of the mean counts in the histogram this 10 μV average wave form represents a very strong synchronization between events associated with the oscillations in the medulla and their oscillatory influence on the motoneurones.

Such extracellular oscillatory wave forms at expiratory sites were interpreted by Cohen (1973) as the extracellular counterpart of the inhibitory oscillations observed intracellularly by Mitchell & Herbert (1971, 1974) in the expiratory neurones of the medulla. This form of synchronous behaviour has been referred to as high-frequency oscillation (h.f.o.) (see Cohen, 1979). On the basis of the very strong link deduced from the data of Fig. 2 we have denoted synchronization with a periodicity in the same range (60–120 Hz) as h.f.o. synchronization.

We have thus defined three different types of synchronization for intercostal motoneurones, namely, short-term synchronization, broad-peak synchronization and h.f.o. synchronization. We wish to ascribe the origins of these three types of synchronization to different sets of input fibres as follows. Only short-term synchronization fits the simple hypothesis of branched presynaptic axons and we show below that there is an association between the presence of short-term synchronization and the respiratory drive. When taken together with previous evidence linking the a.c.e. potential and the respiratory drive (Kirkwood & Sears, 1978) and the measurements of conduction velocity in the following paper (Kirkwood *et al.* 1982), this evidence leads us to suggest that the main input fibres involved in the generation of short-term synchronization are those of the bulbospinal respiratory neurones, which are believed to make monosynaptic connexions to motoneurones (Kirkwood & Sears, 1973; Cohen, Piercey, Gootman & Wolotsky, 1974; Hilaire & Monteau, 1976). For the reasons given above, h.f.o.-synchronization may be confidently ascribed to the presynaptic synchronization of these same neurones and the evidence that follows supports this view. We believe the remaining type of synchronization, broad-peak synchronization, also to be due to presynaptic synchronization, because of the duration of its central peak (± 20 ms or more). Results of other studies suggest that the bulbospinal neurones do not participate in this form of presynaptic synchronization, because the time course of their synchronization corresponds either to h.f.o. or to short-term synchronization only (Vachon & Duffin, 1978; Feldman, Sommer & Cohen, 1980; G. Hilaire, personal communication). The evidence that follows suggests instead that spinal cord interneurones are involved.

The state of the preparation: effects of anaesthesia

Our original observation of the broad peaks was associated with presumed interneurone activity in the spinal cords of some of the preparations which were known to be lightly anaesthetized. In order to make an objective comparison we have compared the duration of the cross-correlation histogram peak to a parameter directly related to the hypothesis of active interneurones and have taken advantage of the relatively large populations of motoneurones which were surveyed by Kirkwood & Sears (1982*b*). They measured multi-unit cross-correlation histograms between the afferent discharges of single muscle spindle afferents and efferent discharges in inspiratory filaments. E.p.s.p.s with polysynaptic components should have longer durations and longer rise times, on average, than those without. Thus cross-correlation histograms involving polysynaptic connexions as well as monosynaptic ones should also have longer durations than those involving only a monosynaptic link. Those preparations with prominent interneuronal activity should give relatively long duration cross-correlation peaks both between the discharges of

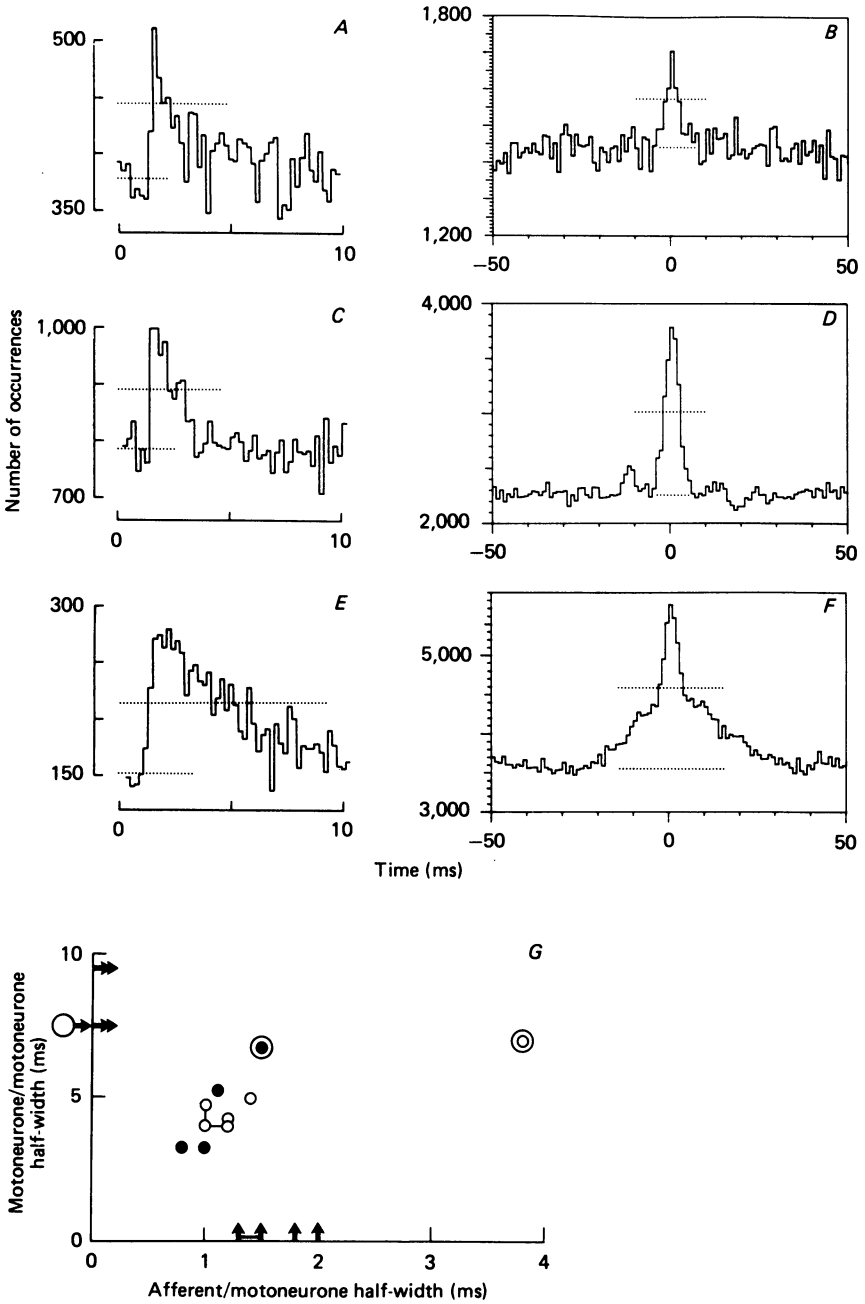

Fig. 3. Comparison between afferent/motoneurone and motoneurone/motoneurone cross-correlation histograms. *A*, *C*, *E*, afferent/motoneurone histograms (bin width 0·2 ms) from the data of Kirkwood & Sears (1982*b*), using multi-unit efferent discharges (all α-efferents of a given filament in each case), each histogram from a different animal. Zero time is the time of the afferent spike in the dorsal root. *B*, *D*, *F*, motoneurone/motoneurone histograms respectively from the same animals. *A*, afferent and efferents in T7; *B*, efferents in T6 (reference spikes) and T7. *C*, afferent and efferents in T6; *D*, efferents in T6 (reference

muscle spindle afferents and motoneurones and between the discharges of paired groups of motoneurones. It should be noted that we are not suggesting that the same interneurones are necessarily involved in the two measurements but that the activity in both sets of interneurones may be similarly effected by anaesthesia and thus the measurements may usefully compare the states of different animals.

In nearly all the animals for which the afferent/motoneurone cross-correlation measurements were made, recordings were also made from at least two inspiratory filaments, including one in the same segment as the afferent. We have therefore constructed motoneurone/motoneurone cross-correlation histograms from all these recordings and measured the half-widths of the peaks in both types of cross-correlation histograms. Examples of the two types of histograms, with the levels used for half-width estimation, are shown in Fig. 3*A–E*. The base lines for the afferent/motoneurone histograms (Fig. 3*A*, *C* and *E*) were calculated as the means of the bin counts before the peaks (Kirkwood & Sears, 1982*b*). The base lines for the motoneurone/motoneurone histograms (*B*, *D* and *F*) were drawn by eye to take account of the respiratory non-stationarity (cf. Fig. 1).

The three afferent/motoneurone histograms (Figs. 3*A*, *C* and *E*), increase in half-width from *A* to *E*. Similarly the half-widths of the motoneurone/motoneurone histograms increase from *B* to *F* (3·5 ms, 5·0 ms and 7·0 ms respectively). It should be noted that weak h.f.o. synchronization is present in *D* and in *F*, which may make the central peak slightly narrower than it otherwise would be by virtue of the two troughs on either side of the central peak (at about half amplitude in *F*). In histograms constructed from the discharges of other filaments in the same preparation as in *F*, the h.f.o. effects were more prominent.

Measurements of this sort from fourteen animals (all anaesthetized with pentobarbitone) are summarized in Fig. 3*G*. Each symbol represents measurements from a single animal with respect to recordings from one intercostal space and a given anaesthetic state (a single recording run), with two exceptions. Firstly, the three points joined together represent three different levels of anaesthesia or CO_2 for the discharges recorded from the same filaments of one animal. Secondly, the two arrows joined by a bar represent two different afferents (different intercostal spaces) in another animal. For three animals, strong h.f.o. synchronization was present, making

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spikes) and T7. *E*, afferent and efferents in T8; *F*, efferents in T7 (reference spikes) and T8. End-tidal CO_2: *A*, *B*, 5·5%; *C*, *D*, 6·3%; *E*, *F*, 7·0%. Dotted lines show base lines and levels for half-width estimations. *G*, half-widths of the two types of histogram plotted together. Each point represents the half-width of the afferent/motoneurone histogram for afferent and efferents within one segment (abscissa) and the average half-width of the two motoneurone/motoneurone histograms between the efferents of this segment and the two adjacent segments, except one point where only one of these motoneurone/motoneurone histograms was available. Different animals, except for the three points joined by lines, which represent three different experimental runs for the same animal. Vertical arrows represent motoneurone histograms where strong h.f.o. synchronization was present and a half-width for the motoneurone/motoneurone histogram was therefore meaningless. Two arrows joined by a bar represent two afferents (different segments) in one animal. ○, weak h.f.o. synchronization; ●, no h.f.o. synchronization; ◎ and ⊙, polysynaptic e.p.s.p.s observed. Horizontal arrow and large circle, polysynaptic e.p.s.p. observed but no half-width measured for afferent/motoneurone histogram. Double horizontal arrows, lightly anaesthetized animals, no afferents recorded.

the measurement of the half-width of the motoneurone/motoneurone histogram peak meaningless. These animals are thus represented only by the measurements of the afferent/motoneurone histogram half-width (arrows on abscissa). In one animal the afferent/motoneurone histogram was too weak to allow the measurement of a half-width but the motoneurone/motoneurone histogram half-width is indicated on the ordinate (arrow plus circle) and is of interest because in this animal polysynaptic components were seen in spike triggered afferent e.p.s.p.s (Kirkwood & Sears, 1982*a*). The motoneurone/motoneurone histogram half-widths from two other lightly anaesthetized preparations are also indicated on the ordinate.

The result is clear. The five lightly anaesthetized animals gave the widest motoneurone/motoneurone histogram peaks. The two afferent/motoneurone histogram peaks from these animals were the widest seen and there was a clear significant positive correlation between the half-widths of the two types of histogram peaks (correlation co-efficient 0·74 for the ten points on the graph; $P < 0·05$). Furthermore, the three animals giving strong h.f.o. synchronization gave afferent/motoneurone histogram peaks with half-widths in the upper part of the range and those with weak h.f.o. synchronization in the upper or intermediate range. Since the activity of spinal cord interneurones is well known to be depressed by anaesthesia, as is h.f.o. synchronization (Cohen, 1973), the results from these fourteen animals taken together are entirely consistent with our hypothesis that the variable width of the motoneurone/motoneurone cross-correlation histogram peaks represents a variable level of synchronized interneuronal activity, strongly dependent on the level of anaesthesia.

This relationship between the state of the preparation, as reflected in motoneurone synchronization and level of anaesthesia, was specifically tested in six preparations by measuring the motoneurone synchronization before and after a supplementary dose of pentobarbitone (see Methods). In all cases the dose of pentobarbitone affected the form of the cross-correlation histogram. Fig. 4 shows four representative examples. In each animal histograms constructed from the recordings of other filaments gave similar results. The first example shows the clearest single effect (Fig. 4*A* and *B*). In this animal a supplementary dose of 6 mg/kg produced a change from a strong broad-peak plus short-term synchronization to short-term synchronization alone in all the combinations of filament recordings that were tested. The end-tidal CO_2 was deliberately increased after the dose of anaesthetic in order to maintain the level of efferent activity approximately constant.

Fig. 4*C* and *D* shows the effect of the same supplementary dose (6 mg/kg) of anaesthetic in an animal showing weak h.f.o. synchronization. Like the broad peak in the previous example the h.f.o. component in the synchronization was also abolished by the anaesthetic, leaving only short-term synchronization. In this case the end-tidal CO_2 concentration was maintained constant.

The general result was always that both broad-peak synchronization and h.f.o. synchronization were reduced by additional anaesthesia. However the change was not always as simple as in these first two examples. Often broad-peak and h.f.o. synchronization were present together. In these instances one or other component sometimes survived the additional anaesthetic. For instance, in Fig. 4*E* the presence of a weak broad peak prevents the troughs between the h.f.o. peaks extending below the base line (cf. Fig. 1*F*). A rather smaller dose of anaesthetic than the previous

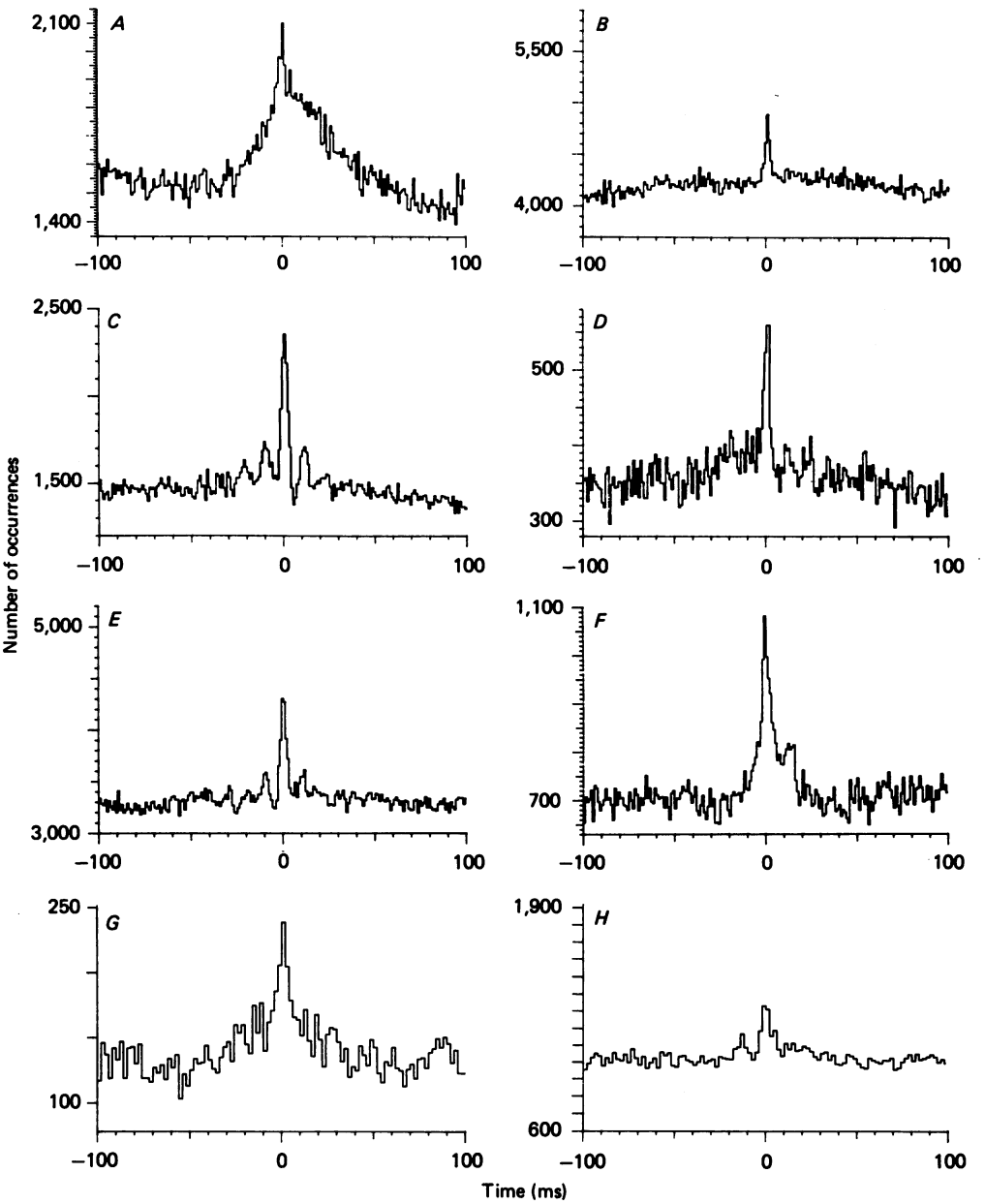


Fig. 4. Effects of additional anaesthesia on motoneurone synchronization. Left hand column (*A*, *C*, *E*, *G*) before, and right hand column (*B*, *D*, *F*, *H*) after a supplementary dose of pentobarbitone. Each pair of histograms represents a different animal, which were all paralysed and artificially respired except the one for *G*, *H*, which was breathing spontaneously. *A*, *B*, efferents in T7 (reference spikes) and T8, supplementary dose 6 mg/kg, end-tidal CO_2 4·7% before and 6·0% after this dose. *C*, *D*, efferents in T6 (reference spikes) and T7, supplementary dose 6 mg/kg, end-tidal CO_2 6·8% for both histograms. *E*, *F*, efferents in T6 (reference spikes) and T7, supplementary dose 4 mg/kg, end-tidal CO_2 6·0% before and 6·4% after this dose. *G*, *H*, histograms (2 ms bin width) constructed as the sums of histograms from efferents in T5 and T6, T6 and T7, T5 and T7 (short experimental runs), supplementary dose 3 mg/kg, end-tidal CO_2 not measured. The two histograms of each pair scaled in proportion to the base line counts.

examples (4 mg/kg) abolished h.f.o. synchronization in this case but left a very clear (though weak) broad-peak component (Fig. 4*F*). The amplitude of the central peak in this example is actually higher after the anaesthetic supplement than before, suggesting that either the broad-peak component or the short-term component had become stronger. It should be noted, though, that in this example the CO_2 was raised only slightly after the additional anaesthetic and the level of activity was considerably reduced. Thus the population of motoneurones was not the same for the two histograms.

The reverse effect is seen in Fig. 4*G* and *H*. In this animal, as in Fig. 4*A* and *B*, a small supplementary dose of anaesthetic (3 mg/kg) abolished the broad-peak component of the synchronization, but this time left a weak h.f.o. component. The noise level in Fig. 4*G* is too high properly to judge whether this level of h.f.o. synchronization was present before the additional dose of anaesthetic was given. However, the P_{A,CO_2} may have risen after this dose and a high level of CO_2 is conducive to h.f.o. synchronization (see next section and Cohen, 1973). This animal was breathing spontaneously and no measurements of CO_2 were made.

It should be noted that the animals illustrated in Fig. 4 were not very much more lightly anaesthetized than those of Sears & Stagg (1976). However, an important difference may have been the over-all state of the animal over the six to eight hours or more of preparation time. At least some of the animals that gave the broad-peak or h.f.o. components were specifically noted as ones which had been maintained fairly lightly anaesthetized, with a high steady blood pressure and a good temperature throughout the preparatory period.

The state of the preparation: effects of CO_2

In the preceding section it was shown how altering the level of anaesthesia changed the form of the synchronization of particular groups of motoneurones. These groups were defined by the discharges recorded from individual nerve filaments. As anaesthesia, and perforce P_{A,CO_2} changed, so each population of motoneurones may have changed its composition. Thus it might be argued that the anaesthetic given to the animal illustrated in Fig. 4*A* and *B* depressed the activity of a sub-group of motoneurones showing broad peak synchronization and the CO_2 that was then given to restore the activity recruited a different sub-group. However, we have no evidence to suggest that there are heterogenous groups of motoneurone represented in a filament. Although under different anaesthetic conditions different regions of the thorax may be recruited in different orders, the recruitment order of α-motoneurones within a given filament recording appears to be relatively constant (see illustrations in Sears, 1963, 1964*a*). For instance, during a breath or as CO_2 increases or as anaesthesia changes the α-spikes in a filament recording are usually recruited in the order small spikes first, then larger spikes. The particular motoneurones recruited and the range of motoneurones within this recruitment order will undoubtedly have varied between the various experimental runs, but we believe, only by the addition of the higher threshold units in the runs with more activity, not by the replacement of some units by others. Thus, if the higher threshold units showed short-term synchronization whereas the low threshold units gave broad-peak synchronization, then the histograms from runs with high activity would give a proportionately lower

strength of broad-peak synchronization than runs with low activity, merely by the averaging effect of the multi-unit cross-correlation histograms.

This effect would be indistinguishable from the effect that could occur in a single motoneurone if, at a low level of activity, a high proportion of the unitary e.p.s.p.s were derived from individual input fibres whose discharges were themselves synchronized, but at a high level of activity the additional inputs were unsynchronized and therefore contributed to the motoneurone synchronization only via their branched axons. We will refer to this latter possibility as 'dilution' of a synchronized input by a non-synchronized one.

In some ways it is not important which of these two possibilities occur; either represents a change in the over-all input to the pool of active motoneurones. Nevertheless, investigating the synchronization at different levels of activity does help identify the sources of the different inputs presumed to be involved. We therefore varied the P_{A,CO_2} and constructed cross-correlation histograms at different levels of activity for preparations showing the different forms of synchronization.

Kirkwood & Sears (1978) showed that the fast rising phase of the a.c.e. potential was proportional to the inspiratory drive on a given motoneurone. They therefore deduced that the main input responsible for the a.c.e. potential and the short-term synchronization was generated by the axons transmitting the inspiratory drive to the motoneurones. If this is correct, then in animals showing only short-term synchronization, increasing the P_{A,CO_2} should only add more of this drive and the cross-correlation histogram should remain qualitatively the same.

This was found to be so in both of the animals in which the test was made and where only short-term synchronization was present (end-tidal CO_2 concentration changes 6·2–9·1 % and 6·0–9·2 %). Cross-correlation histograms from one of these animals are shown in Fig. 5*A* and *B*. The peak in the histogram *B*, at high CO_2 is slightly shorter in duration than that in *A* but the effect is small. This could have several explanations, such as a change in the relationship between the shapes of individual e.p.s.p.s and the raised probabilities of firing they create as the synaptic noise increases with the increased respiratory drive, or a change in the distribution of conduction velocities of motoneurones or pre-motor neurones involved. For our present purposes it is a second order phenomenon, sometimes observed. The reverse, a wider peak at high CO_2, was never seen.

In addition to measuring the durations of these peaks we have estimated the strengths of the short-term synchronization as in Sears & Stagg (1976), by the factor k, defined as the ratio of peak counts to the base-line counts. In this paper we have always measured k from histograms of 1 ms bin width and estimated the base-line counts by eye so as to exclude the respiratory non-stationarity, as in Fig. 3*B*, *D* and *F*. In the example of Fig. 5*A* and *B*, k changed from 1·11 to 1·09 for an end-tidal CO_2 change of 6·0–9·2 %. This was a general result which was true for five out of six pairs of filaments where a reasonably accurate assessment of k could be made (filaments in the same segment or adjacent segments) and where no other form of synchronization was present. The sixth example gave a small rise in k with CO_2. The mean reduction in $k-1$ was 27 % (S.D. 15 %). There could be several explanations for the reduced amplitudes with higher CO_2 levels but the most interesting aspect of the result is that the change is small despite a considerable recruitment of motoneurones

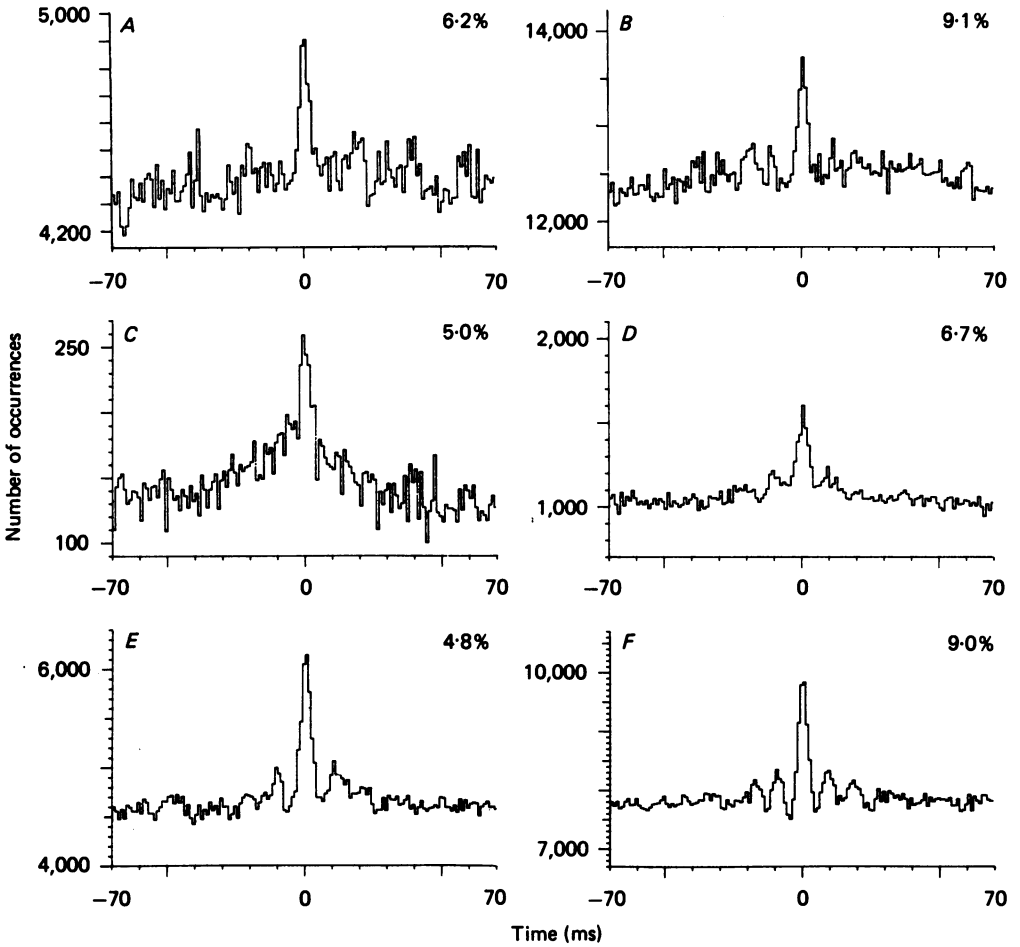


Fig. 5. Effects of CO_2 on motoneurone synchronization. Each pair of histograms (*A*, *B*; *C*, *D*; *E*, *F*) from a different animal at the end-tidal CO_2 levels indicated. *A*, *B*, histograms constructed as the sums of histograms from efferents in T6 and T7, T7 and T8, T8 and T9. *C*, *D*, efferents in T8 (reference spikes) and T9. *E*, *F*, efferents in T6 (reference spikes) and T7. The two histograms of each pair scaled in proportion to the base line counts.

at the higher CO_2 levels. This suggests that either high and low threshold units are almost as strongly synchronized as low threshold units are with each other, or the high threshold units are more strongly synchronized, so that the over-all strength of synchronization was largely unchanged. Hence either all the motoneurones have a similar proportion of common inputs from the source represented by short-term synchronization or, if low and high threshold motoneurone have few common inputs, then high threshold units must have more common inputs between themselves.

When the other forms of synchronization were present, increasing the level of CO_2 made more dramatic changes. Fig. 5*C* and *D* is an example where at 5·0 % CO_2 a broad peak plus a narrow peak were present. Increasing the end-tidal CO_2 to 6·7 % changed the histogram to a narrow peak plus weak h.f.o. synchronization and only a vestige

of the broad peak, which is detectable because the troughs on either side of the main peak do not reach the base line.

The most obvious interpretation of this change is that increasing the level of CO_2 has increased the descending respiratory drive from the medulla. The two types of synchronization which we believe are related to this input (short-term synchronization and h.f.o.) are thus exaggerated with respect to the broad-peak synchronization which we have already shown to be linked to the activity of interneurones in the spinal cord. This exaggeration could most simply occur if the respiratory input 'diluted' a non-respiratory input which generated the broad-peak synchronization, as described above. However, other explanations are possible: (i) the descending respiratory drive could directly inhibit the spinal cord interneurones believed to be responsible for the broad-peak synchronization, or could inhibit their terminals presynaptically; (ii) high CO_2 could depress the activity of these interneurones directly (cf. Kuno & Pearl, 1960); (iii) if the interneurones have an excitatory respiratory input, the increased respiratory drive could make their discharges less synchronized.

In another animal, the synchronization at 4·0 % CO_2 (Fig. 5*E*) had the same form as the previous example at high CO_2, with signs both of weak h.f.o. and weak broad-peak components. Increasing the CO_2 to 9·0 % for this animal had the effect of maintaining the narrow central peak, exaggerating the h.f.o. and eliminating the broad peak. (Note that the h.f.o. troughs now go below the base line.) The h.f.o. period also decreased from 11 to 9 ms.

Results similar to these two examples (Fig. 5*C–F*) were seen in a large number of histograms from four animals, i.e. a general reduction in the proportion of broad-peak synchronization in favour of short-term or h.f.o. synchronization as the end-tidal CO_2 increased.

Decerebrate preparations

Two preparations decerebrated under thiopentone sodium were tested for synchronization of inspiratory discharges. One gave the h.f.o. synchronization shown in Fig. 2. The other, tested only a few hours after the anaesthetic, gave clear short-term synchronization ($k = 1·25$, half-width of peak 3·5 ms, for two filaments in the same segment).

Effects of dorsal root section

The thoracic dorsal roots contain afferents that excite motoneurones monosynaptically in the same and adjacent segments (Sears, 1964*b*; Kirkwood & Sears, 1982*a*, *b*) and polysynaptically with a wider distribution (Downman, 1955; Aminoff & Sears, 1971).

On *a priori* grounds it seemed likely that dorsal root section, by altering excitability of spinal cord pathways (cf. Kirkwood *et al.* 1981*b*), might alter the balance of the different inputs to the motoneurones and thus favour one or other type of synchronization. However, our results to date suggest that is not so. Two experiments were performed in which data for cross-correlation were recorded before and after section of the dorsal roots of the three segments from which the filaments were derived. Both of these animals demonstrated only short-term synchronization. In both of the animals this persisted after dorsal root section.

However, our principal evidence concerning the influence of dorsal root afferents is that many of the experiments described above were performed with the ipsilateral dorsal roots cut in up to five segments, always including the ones used for efferent recordings (Kirkwood & Sears, 1982*a*, *b*; Kirkwood *et al.* 1981*b*). An approximately equal number of the experiments were performed with the roots intact. All the effects described in the preceding sections were observed in both types of preparation. Our conclusion is that afferent inputs via the dorsal roots are not necessary for any of the three types of synchronization described here, but with the proviso that section of contralateral afferents or afferents in more distant segments was not investigated.

Effects of spinal cord lesions

In contrast to the negative results from the peripheral deafferentation just described, partial acute central deafferentation had strong effects on the forms of the cross-correlation histograms. Central deafferentation was achieved by means of acute partial or full transection of the cord above or below the segments which provided the efferent discharges. For three animals the aim was to make an ipsilateral hemisection above the segments of interest so as to remove the main part of the descending respiratory drive. On histological examination it was later found that all these hemisections were incomplete (cf. Fig. 6). Some ipsilateral ventromedial white matter had survived, together with a portion of the dorsal column. Three further animals, intended as controls for experiments on chronic partial deafferentation (Kirkwood *et al.* 1981*a* and in preparation), were intended to have ipsilateral hemisections above and below the segments of interest. Again, on histological examination these lesions were found to be incomplete. A seventh animal was given a complete transection of the cord below the segments of interest, which was also a lesion added to two of the previously double lesioned animals. In one of these two animals an intermediate type of lesion which consisted of an ipsilateral hemisection plus section of some contralateral dorsal columns plus ventromedial white matter below the segments of interest was made before the total transection.

In all six animals where a lesion was made above the segments of interest the ipsilateral efferent α-discharges below the lesion were abolished at eupnoeic levels of CO_2. Nevertheless, in five of these animals inspiratory-phased α-discharges could be evoked, but with their thresholds for activation raised to between 7 and 9·5 % end-tidal CO_2, instead of a normal value of 3·5–4·5 %. The maximum discharge was always much less than before the lesion(s). Often only one or two α-units remained active in each filament and then only at the peak of inspiration. This result confirms that the main respiratory drive to intercostal motoneurones descends ipsilaterally in the cord (see Cohen, 1979 for review). The drive that remained after the lesions could have been derived in part from the white matter that survived the lesions (the lesion in the animal in which no α-discharges could be evoked was the most complete) and partly from the opposite side of the cord. This latter component is directly indicated by the fact that the contralateral discharges below the lesions were somewhat reduced by the lesions and our knowledge that respiratory phased discharges are present at eupnoeic levels of CO_2 in animals with ipsilateral chronic hemisections known to be complete (Kirkwood *et al.* 1981*a*).

The general result for the cross-correlation measurements was that all lesions

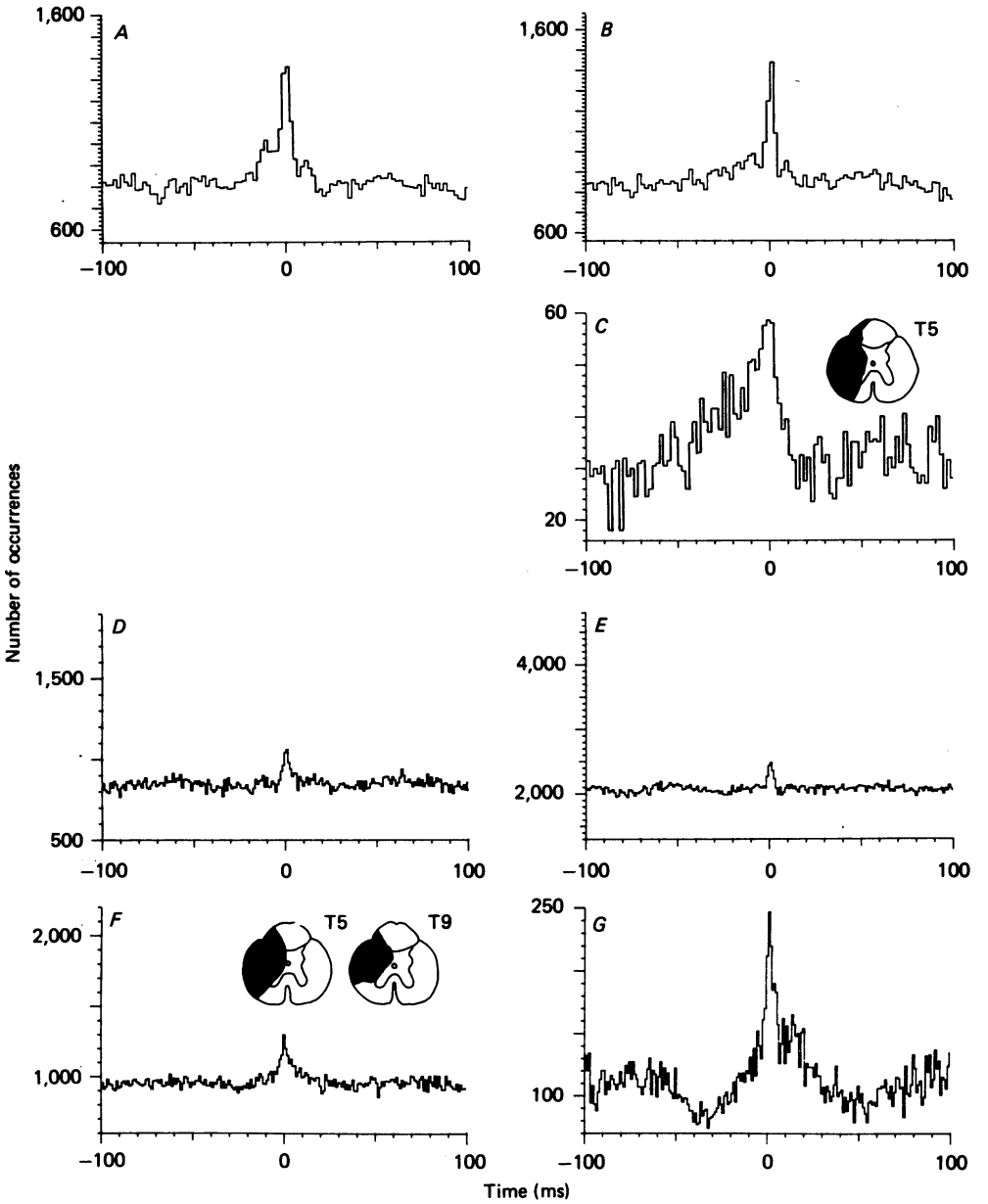


Fig. 6. Effects of acute spinal cord lesions on motoneurone synchronization. *A–C*, histograms (2 ms bin width) from efferents in T6 (reference spikes) and T7. *A*, *B*, end-tidal CO_2 7·0 % and 9·5 % respectively before the lesion shown in *C* (T5); *C*, end-tidal CO_2 9·5 % after the lesion. *D–G*, histograms from another preparation; both sets of efferents in T7. *D*, *E*, end-tidal CO_2 6·0 % and 9·2 % respectively before lesions at T5 (left inset in *F*) and T9 (right inset in *F*); *F*, end-tidal CO_2 7·7 % after these lesions; *G*, end-tidal CO_2 7·2 % after a further lesion (total transection at T10). *A–C* and *D–G* scaled in proportion to the base line counts.

promoted broad peak synchronization, in some cases producing an effect considerably stronger than light anaesthesia. Fig. 6 shows two examples. *A–C* are from a cat which showed short-term synchronization together with a mixture of weak h.f.o. and weak broad-peak synchronization. (Fig. 4*C* and *D* and Fig. 5*C* and *D* come from the same animal.) Histograms from two different levels of CO_2 before the lesion are shown (Fig. 6*A* and *B*) and from one level of CO_2 after the lesion (*C*), which was at T5 (inset in *C*). The pre-lesion cross-correlation peaks of this animal were strong and in some cases had a considerable duration, particularly at the lower CO_2 levels (cf. Fig. 5*C*). Nevertheless, both the strength and duration of the central peak were considerably increased following the lesion, despite the high level of CO_2.

Fig. 6*D–G* shows another series of histograms, this time from an animal that showed only short-term synchronization before the lesion (*D* and *E*). Following lesions in two segments, T5 and T9 (see insets), the histogram *F* was obtained. It should be noted that, probably because the upper lesion was relatively small, a lower level of CO_2 was sufficient to evoke a suitable motoneurone discharge here than was used in the example of Fig. 6*C*. Again the histogram *F* has a peak which is both stronger and of longer duration than those measured before the lesions. The amplitude and duration themselves are not remarkable, being similar to those for a lightly anaesthetized animal (the amplitude is about the same in Fig. 1*D* and the duration is slightly less), but they represent a considerable change for this preparation. Moreover, a very large change was apparent when the other main category of lesion was subsequently made for this same preparation. Fig. 6*G* shows the histogram obtained from the same two filaments following additional total transection of the cord at T10. The effect was to produce a very long duration peak with an amplitude greater than in any of the non-lesioned animals.

The effects of the upper lesion are readily understood in terms of our main hypothesis, that the broad-peak synchronization represents the synchronized activity of spinal cord interneurones and the short-term synchronization is mainly generated by the ipsilateral descending respiratory input. Removing a large part of this input reduces the 'dilution' or other effects already described but, since threshold for the motoneurones that contribute to the histogram was still achieved, albeit at an elevated level of CO_2, then it is likely that this respiratory input had been replaced by some other excitatory input. We presume that this other input was provided by spinal cord interneurones released from descending controls (Downman, 1955; cf. Sherrington & Sowton, 1915; Eccles & Lundberg, 1959), but we do not know whether these interneurones have respiratory patterns of activity or not. They may or may not be the same ones that are active in lightly anaesthetized animals.

The strong effect of the lower transection was not expected, but was repeated on two other occasions. We again presume it represents the release of interneurones, this time from tonic ascending controls. The effect may be stronger merely because a total transection releases more interneurones than a partial transection. A total transection was possible below the segments of interest without abolishing the motoneurone discharges, which was not possible for the upper lesions. The one animal given an intermediate sized lesion below the segments of interest, before the total transection, showed an intermediate effect. Fig. 7 illustrates the effects of total transection alone. *A* and *B* show that before the lesion broad peak plus short term synchronization were

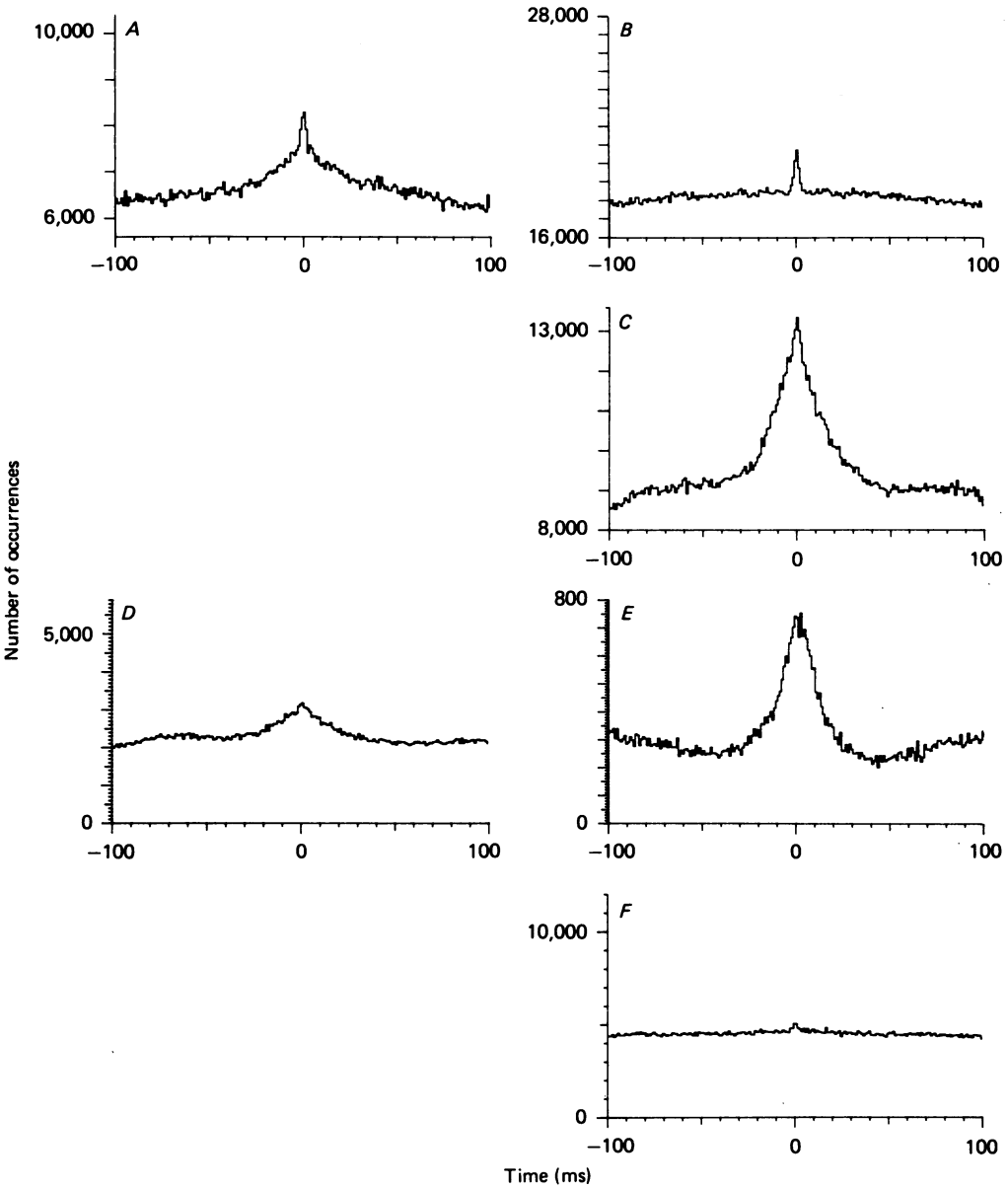


Fig. 7. Effects of acute spinal cord lesions on motoneurone synchronization. All histograms from one preparation. *A–C*, histograms constructed as sums of histograms from afferents in T4 and T5, T5 and T6, T6 and T7, T7 and T8. *A*, end-tidal CO_2 4·7% before supplementary dose (6 mg/kg) of anaesthetic; *B*, end-tidal CO_2 6·0% after this dose, both of these before the lesion; *C*, end-tidal CO_2 6·4% after the lesion (total transection at caudal T9). *D–F*, all after this lesion, efferents in T5 and T6; *D*, end-tidal CO_2 6·4% before a supplementary dose (12 mg/kg) anaesthetic; *E*, end-tidal CO_2 6·4% after this dose; *F*, end-tidal CO_2 9·5% (recording taken a few minutes after *E*). *A–C* and *D–F* scaled in proportion to the base line counts. Note scale starts at zero for *D–F*.

present when the animal was lightly anaesthetized (*A*), but only short term synchronization (*B*) following an anaesthetic supplement. After the lesion (*C*) a broad peak was present with an amplitude of about three times what it had been in the lightly anaesthetized state.

The histograms used for *A–C* are the sum of the individual histograms drawn from four pairs of recordings made in adjacent segments, whose individual histograms consistently showed the same effect. They are presented in this way because the high signal/noise ratio thereby achieved shows clearly the distinction between the separate components.

The broad-peak synchronization seen after the lower lesion was not only strong, but was also resistant to anaesthesia. Fig. 7*D* and *E* compares histograms from one of the individual pairs of filament discharges (T5 and T6) before (*D*) and after (*E*) a relatively large supplement of anaesthesia (12 mg/kg) given in the post-lesion state. In this particular example the strength of the synchronization is actually increased by this anaesthetic dose, which was strong enough to depress the activity of all the filaments from T4 to T8 so much that the only pair of discharges suitable for correlation was the pair T5, T6. This apparently anomalous result (stronger synchronization with extra anaesthesia) may be explained by consideration of the cross-correlation histogram between these two filament discharges at high CO_2 (Fig. 7*F*). Although the strong broad peak had already survived a large dose of anaesthetic it was severely depressed by a high level of CO_2. This is different from the animals in which an upper lesion had been made and where a strong broad peak was present at a high level of CO_2 (e.g. Fig. 6*C* and *G*). Our interpretation of this is that with the lower lesion alone, the respiratory input still has access to the motoneurones and so either the 'dilution' effect, that is the swamping of the local inputs by the non-synchronized descending respiratory inputs, or the inhibition or desynchronization of interneurones by the respiratory drive can still take place. When the upper lesion is present neither of these are possible. The exaggeration of the strong broad peak in Fig. 7*E* thus may represent the converse, that is the removal of the 'dilution' or other effects via depression of the respiratory drive by extra anaesthesia. The implication is that the interneuronal activity released by the lower lesion must itself be resistant to anaesthesia, perhaps because this activity is much greater than that in a normal lightly anaesthetized animal. From the example of Fig. 7 we still cannot rule out that this activity might be directly depressed by CO_2 but in the two cats where a lower transection was added in the presence also of an upper lesion, the strong synchronization seen after the lower lesion persisted at high CO_2 (e.g. Fig. 6*G*), up to 9·6% in one case.

Measurements on expiratory discharges

Measurements were made in four cats from internal intercostal (expiratory) nerve filaments prepared in the same way as the inspiratory ones and from expiratory e.m.g. records in one cat. Central peaks with a variety of strengths and durations were seen in the histograms, similar to those for the inspiratory discharges, though with no example of h.f.o. synchronization. H.f.o. synchronization has not been reported for expiratory discharges by other workers.

The correspondence between these histograms and the cross-correlation histograms from the inspiratory discharges was not exact, but the general principles seemed to

be upheld. For instance, Fig. 8*A* shows a histogram derived from two expiratory filaments in the same animal and at approximately the same level of CO_2 as the inspiratory filaments represented in Fig. 6*D*. In place of the short-term synchronization, there is a relatively strong broad peak. This may not be a real difference; the animal was more lightly anaesthetized when the expiratory discharges were recorded.

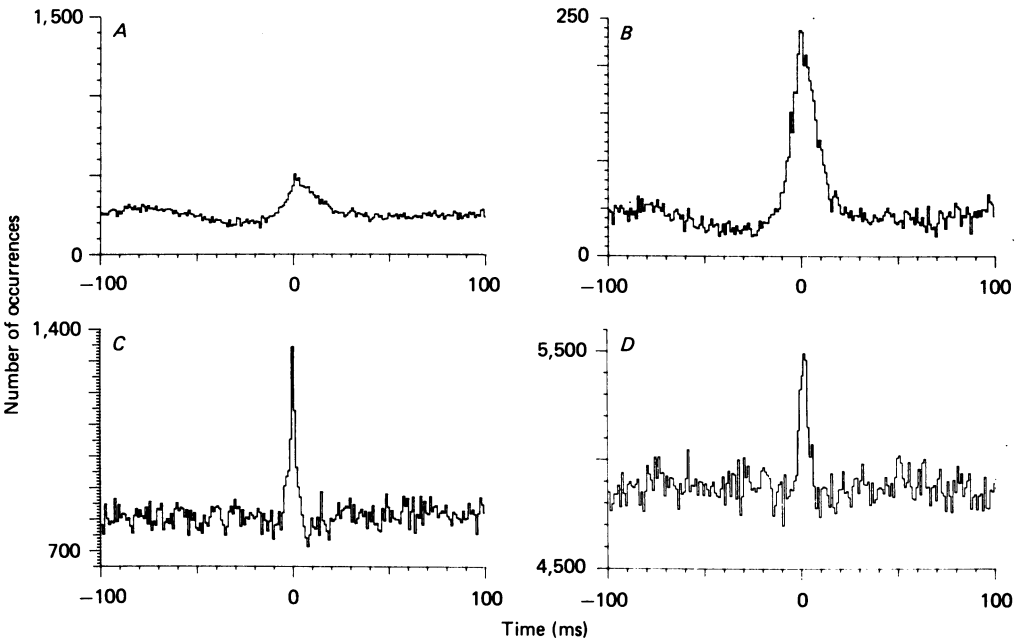


Fig. 8. Cross-correlation histograms from expiratory discharges. *A*, *B*, efferents in T6 (reference spikes) and T7; *A*, rhythmic respiratory discharges, end-tidal CO_2 5·8 %; *B*, same filament recordings but tonic discharges, end-tidal CO_2 2·4 %. *C*, different preparation, efferents in T7 (reference spikes) and T8, tonic discharges, end-tidal CO_2 1·8 %. *D*, a third preparation (decerebrated under halothane anaesthesia), rhythmic discharges, end-tidal CO_2 4·0 %, spontaneous respiration, efferents recorded as e.m.g. signals (a few motor units in each recording channel) from T6 (reference spikes) and T8 (data from the recordings of Bainton *et al.* 1978). *A*, *B* scaled in proportion to the base line counts.

The particular interest of this recording, however, is the comparison with the histogram derived from the tonic discharges of the same filaments in hypocapnic apnoea (2·4 % end-tidal CO_2) which is shown in Fig. 8*B*. Broad-peak synchronization of the same duration is still present, but with a markedly increased strength.

We studied hypocapnic apnoea because this provides a further way of dissociating descending respiratory inputs to the motoneurones from others. For hypocapnic apnoea in general, especially in light anaesthesia, expiratory bulbospinal neurones still provide some excitation of motoneurones (Bainton *et al.* 1978), but at 2·4 % end-tidal CO_2 the bulbospinal neurones are either silent or almost so (Bainton & Kirkwood, 1979), and the discharge of the motoneurones must be maintained almost entirely by other inputs (cf. Sears, 1964*b*). Once again, the results of diminishing the respiratory inputs is to favour broad-peak synchronization.

It should also be mentioned that in one other animal where internal intercostal

filament discharges were investigated in hypocapnic apnoea, only short-term synchronization was ever seen, although the peak was stronger than was usual for the inspiratory discharges (Fig. 8*C*). In this animal the cross-correlation histogram was almost identical at other levels of CO_2. We presume that the non-respiratory inputs maintaining the discharge here were not synchronized with each other. Therefore, removing the 'diluting' respiratory input did not change the proportion of synchronized *versus* non-synchronized inputs. This experiment is thus the direct converse of increasing the CO_2 in an animal which showed only short-term synchronization for the inspiratory discharges. This procedure, as we showed earlier, had no qualitative effects.

Of the three other animals investigated, one gave a broad peak (half-width 10 ms); the other two, both decerebrate, gave relatively weak, short-term synchronization, as in Sears & Stagg (1976). One of these is illustrated in Fig. 8*D*.

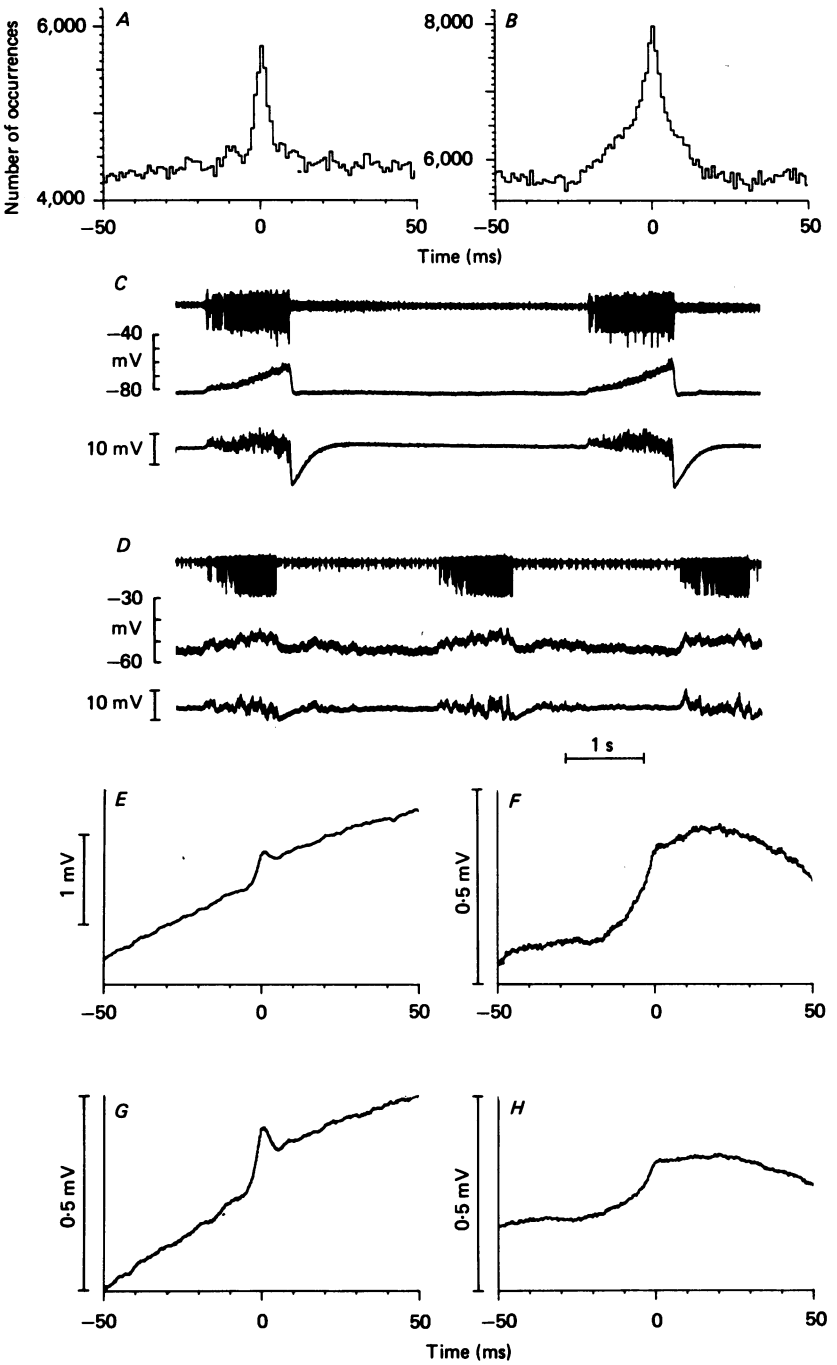
Intracellular recordings

Intracellular recordings from inspiratory (external intercostal) motoneurones were made in eight animals, in addition to the animals investigated for a.c.e. potentials by Kirkwood & Sears (1978). One of the purposes of these recordings was to look for intracellular evidence for the operation of different inputs, corresponding to the different states of the preparations revealed in the cross-correlation histograms. One feature, polysynaptic e.p.s.p.s from individual spindle afferents in the lightly anaesthetized animals, has already been described (Kirkwood & Sears, 1982*a*). The features we were looking for were differences in the proportions of the depolarization that was derived from respiratory sources (identified by the appropriate periodicity and time course) or from other sources; differences in the synaptic noise, corresponding to the presynaptic synchronization we associate with broad peak synchronization and differences in the a.c.e. potential, which is the averaged membrane potential of one motoneurone before and after the time of a spike in another motoneurone (Kirkwood & Sears, 1978).

Full analysis of these recordings is beyond the scope of this paper. Here we merely wish to report that apparently consistent differences were seen in all these features between those animals displaying broad-peak synchronization and those displaying short-term synchronization. No animals with strong h.f.o. synchronization were investigated. Examples typifying the differences are shown in Fig. 9.

This Figure was made from the recordings in two animals, one which displayed short-term synchronization plus only very faint signs of broad-peak and h.f.o.

Fig. 9. Comparison between intracellular observations and motoneurone synchronization. *A*, *B*, cross-correlation histograms from different animals, efferents in T6 (reference spikes) and T7 for both animals. *C*, data recorded from the same animal as *A*; top trace, efferent discharge from filament recording in T7; middle trace, intracellular record from inspiratory motoneurone in T7 (d.c.); bottom trace, high-pass filtered version of middle trace. *D*, data recorded from the same animal as *B*; traces as *C*, filament and motoneurone in T6. Note that the d.c. record is at a different gain for *C* and *D* but the high-pass filtered signal is at the same gain. *E*, *F*, a.c.e. potentials for the cells and filament discharges illustrated in *C*, *D* respectively (see text). *G*, average a.c.e. potential for six motoneurones for the same animal as *A*, *C*, *E*. *H*, average a.c.e. potential for five motoneurones for the same animal as *B*, *D*, *F*.


A
B
6,000
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0
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Time (ms)
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synchronization (Fig. 9*A*), and another where a broad peak was the dominant feature of the cross-correlation histogram (Fig. 9*B*). Fig. 9*C* and *D* includes intracellular recordings from inspiratory motoneurones in the first and second animal, respectively. Both of the two cells fired spikes. The first (*C*) depolarized by 2 or 3 mV soon after this record was taken, resulting in the occurrence of spikes just at the end of inspiration in all subsequent cycles. (This cell is also illustrated in Kirkwood & Sears, 1982*b*, Fig. 10) The second cell fired spikes during each inspiration soon after penetration, but the cell then depolarized to the membrane potential shown in Fig. 9*D* at which it stabilized, presumably with the spike mechanism inactivated. Now, although no reliance can be put on the absolute values of membrane potential, it is quite clear that when these cells were firing, threshold was reached in quite different ways in the two cells, despite the over-all respiratory output as represented in the filament discharge (upper trace) being similar for both animals. In the first one (*C*) the only synaptic activation appeared to be during inspiration, when the depolarization progressed smoothly to its peak (the central respiratory drive potential, c.r.d.p.: Sears, 1964*b*). The synaptic noise increased steadily during inspiration and was absent during expiration. The apparent absence of a hyperpolarizing inhibitory phase (Sears, 1964*b*) may be a consequence of deep anaesthesia or of closeness of the membrane potential to the inhibitory reversal potential. In contrast, the second cell showed a smaller, more irregular c.r.d.p. and the synaptic noise (seen most reliably in the lowest trace of each record) had a coarser texture than in the first cell and was also present in expiration.

The most significant difference between the two cells was the different character of the synaptic noise and the consequent irregular (*D*) rather than smooth (*C*) progression of the inspiratory depolarization. Amplitudes of c.r.d.p.s. vary a great deal between cells and so the small sample we have does not give a very reliable measure of this feature. The synaptic noise in *D* was typical of five cells sampled in that animal. The c.r.d.p. was the largest. The cell illustrated in *C* was also the one which gave the largest c.r.d.p. of the six cells tested in its animal. In that animal, the amplitude of the synaptic noise in the different motoneurones increased with the amplitude of the c.r.d.p., so this cell also showed the most synaptic noise. The gain for the high-pass filtered records (lowest traces in *C* and *D*) are the same. It is worth noting that the peak-to-peak amplitude of the synaptic noise is larger in *D* than in *C*, despite the c.r.d.p. being larger in *C* than in *D*.

The ramp-like form of the c.r.d.p. in *C* is unusual for intercostal motoneurones (cf. phrenic motoneurones: Berger, 1979) but was typical for this experiment. A more common profile is illustrated in Fig. 2 of Kirkwood & Sears (1978) for another animal which also showed only short-term synchronization. In this other example the synaptic noise also incremented gradually during inspiration, without the coarse texture evident in Fig. 9*D*.

Such coarse-textured synaptic noise, especially when it continues in expiration, is exactly what we had expected for animals showing broad-peak synchronization, on the hypothesis that this synchronization arises from non-phasic synchronized inputs. In order to confirm the connexion between the synaptic noise and the motoneurone synchronization, we measured a.c.e. potentials for most of the intracellular recordings. Because we were expecting long-duration components of the a.c.e. potentials, corresponding to the broad peaks of the cross-correlation histograms, we modified

our procedure slightly from that described in Kirkwood & Sears (1978). Instead of averaging the high-pass filtered signal of membrane potential, the d.c. record was used instead.

The resulting a.c.e. potentials for the cells illustrated in Fig. 9*C* and *D* are shown in Fig. 9*E* and *F* respectively. The form of the a.c.e. potential in *E* is exactly that of the majority of the cells previously reported (Kirkwood & Sears, 1978), namely a steep rising phase, starting between −5 and −3 ms and ending in a relatively sharp peak at +1 ms.

This feature is superimposed on a steadily rising base line, which is merely the averaged result of the steadily rising membrane potential during inspiration (Fig. 8*C*). The slope of the base line in the average is about 16 mV/s, which compares well with the slope of the individual depolarizations in Fig. 8*C*. In our previous descriptions (Kirkwood & Sears, 1978) the base line had instead a negative slope, due to the high-pass filtering used then. When allowance is made for the sloping baseline of Fig. 8*E* the amplitude of the fast rising phase is 293 μV. The average amplitude of the inspiratory phase of the c.r.d.p. was 14 mV. These figures are of interest because they justify the extrapolation used in the calculation of Kirkwood & Sears (1978) which gave an a.c.e. potential of 225 μV for a 10 mV c.r.d.p.

The a.c.e. potential for the other cell (Fig. 9*F*) has a rather different form. The principal feature is again a rising phase immediately preceding zero, but in this instance the foot is between −20 and −16 ms and the peak at about +1 ms is not very well defined. We cannot say whether or not there is a clear base line trend. When investigated over a longer time the averaged potential had a positive slope (about 4·5 mV/s) from at least −100 ms and a negative slope of similar magnitude out to at least +100 ms.

The a.c.e. potentials of Fig. 9*E* and *F* were typical of their respective preparations, although both were the largest in amplitude. For comparison we have averaged the a.c.e. potentials from the six cells of the first animal (mean inspiratory c.r.d.p. amplitude 5·6 mV) (Fig. 9*G*) and those from the five cells of the second (mean inspiratory c.r.d.p. amplitude 3·1 mV) (Fig. 9*H*).

Comparisons between the a.c.e. potentials of the form seen in Fig. 9*E*, *G* and the corresponding short-term synchronization have already been made; the two phenomena fit well the hypothesis that they originate in the branching of presynaptic axons whose individual discharges are not synchronized (Kirkwood & Sears, 1978). The a.c.e. potentials of Fig. 9*F* and *H* and the corresponding broad-peak synchronization are not so easily analysed. This is partly because there is no clear separation between a baseline effect which may be ascribed to the c.r.d.p. and events of shorter duration, but also because we believe presynaptic synchronization may be involved. The only alternative explanation to presynaptic synchronization would be unitary e.p.s.p.s of very long duration, 20 ms or more, which have not been reported for motoneurones. If one assumes presynaptic synchronization many possibilities become open, for instance, different time courses of that synchronization resulting from common inputs, or the presence of interneurones with time structure in their own discharges, such as bursts of spikes. Such analysis must await further investigation. It is, however, clear from the a.c.e. potentials of Fig. 9*F* and *H* that the broad-peak synchronization of Fig. 9*B* is related, via the long duration features of the a.c.e. potential, to the coarse texture of the synaptic noise of cells like that in Fig. 9*D*.

DISCUSSION

Detailed arguments to support our conclusions have mostly been presented under Results. Here we concentrate on the general aspects. Intercostal motoneurones may exhibit different forms of synchronization, the dominant form being a property of a generalized state of the preparation, as determined by a variety of factors such as anaesthesia, P_{A,CO_2}, decerebration or spinal cord lesions. Of the three forms of synchronization described, h.f.o. synchronization is the least interesting for the present discussion. This phenomenon almost certainly is a property of the respiratory neurones of the medulla (see Cohen, 1979) and thus largely outside the scope of this paper. Its main use here is that it was observed to be promoted or depressed in parallel with short-term synchronization when P_{A,CO_2} was changed. Thus the observations of h.f.o. synchronization strengthen the associations we have deduced between short-term synchronization and the respiratory input to motoneurones.

The association between broad-peak synchronization, light anaesthesia and polysynaptic connexions is clear and hence it seems safe to associate broad-peak synchronization with interneurone activity. We have deduced that the interneurone activity involves presynaptic synchronization because the broad peaks in the cross-correlation histograms and the corresponding a.c.e. potentials do not correspond to the predicted time courses of Kirkwood & Sears (1978) which were derived from the assumption that such presynaptic synchronization was absent. The interpretation that broad peaks such as these originate in presynaptic synchronization is not new (e.g. Arnett, 1975; Arnett & Spraker, 1981; Sears & Stagg, 1976), but the separation of the effects of the different inputs by the experimental manipulations we have employed here has not been achieved before. The nearest compatible results are those of Noda, Manohar & Adey (1969) and Noda & Adey (1970), who demonstrated large changes in the strength of synchronization of neurones in the hippocampus and the association cortex, respectively, with different behavioural states of unanaesthetized cats. However, their histogram peaks, in particular those obtained from the cortex, showed a very wide range of durations (cf. Holmes & Houchin, 1966) and could not confidently be ascribed to particular inputs.

It is worth considering in more detail which neurones are involved in generating short-term synchronization. The association we have shown here between the respiratory drive and the occurrence of short-term synchronization is not strong enough on its own to support the conclusion that short-term synchronization is derived only from the neurones providing this input. However, this association must be viewed together with the proportionality between the amplitudes of the inspiratory c.r.d.p. and the a.c.e. potential (Kirkwood & Sears, 1978). Moreover, the appearance of the c.r.d.p.s such as the one shown in Fig. 9*C* suggests strongly that in the moderately deeply anaesthetized animals the respiratory input is providing the great majority of the depolarization in the intercostal motoneurones of these animals.

The only other input definitely known to be active in these preparations (Critchlow & Euler, 1963) and common to motoneurones of more than one segment (Sears, 1964*b*) is that from muscle spindle afferents. Taking estimated values for the numbers of spindle afferents of large diameter, their projection frequencies to motoneurones and their firing rates from the data of Kirkwood & Sears (1982*a*, *b*), a figure of around

1500 per second, unitary e.p.s.p.s. of about 100 μV in amplitude may be calculated. As in the calculations of Sears (1977), this corresponds to a steady depolarization of only about 1·2 mV compared to (say) 12 mV which may be assumed for the difference between resting potential and threshold. Thus on independent grounds the monosynaptic input from muscle spindles is not likely to provide more than 10% of the input to a firing external intercostal motoneurone under these conditions.

It is therefore quite reasonable that loss of this input following dorsal root section should have no detectable effect on the short-term synchronization of moderately deeply anaesthetized preparations. It is not that the muscle spindle afferent axons that diverge to synapse on different motoneurones do not contribute to the short-term synchronization within a segment or between adjacent segments, only that their contribution is small, corresponding to the small contribution of these axons to the over-all depolarization of the motoneurones. We can thus safely conclude that the short-term synchronization seen in moderately deeply anaesthetized animals is generated by the axons which transmit the respiratory drive. The measurements presented in the following paper (Kirkwood *et al.* 1982) then go further in identifying that input as the monosynaptic connexion from bulbospinal neurones.

For animals in other states, such as lighter anaesthesia, other neurones with axons which diverge to synapse on motoneurones of different segments will also contribute to the short-term synchronization. It is quite likely for instance that these will include some of the interneurones which we are suggesting generate the broad-peak synchronization. Thus these neurones may contribute to both the narrow and the broad peaks of histograms such as Fig. 1*C* and *D*, just as the respiratory input fibres contribute to both the narrow central peak and the h.f.o. components in the histograms such as Fig. 1*E* and *F*. We have no way of identifying which interneurones are involved in generating broad-peak synchronization. We cannot be sure they are in the spinal cord; our only evidence is the association between this form of synchronization and the polysynaptic e.p.s.p.s from muscle spindle afferents and the similarity between the broad peaks in the normal animals and in those with spinal cord lesions, where the inference is strong that the interneurones concerned must be in the thoracic cord.

There is very little direct evidence for the properties of interneurones in the thoracic cord (but cf. Sumi, 1963*b*; Guilbaud, Benelli & Besson, 1977) although there is plentiful evidence for interneuronal *pathways* (Downman, 1955, Sumi, 1963*a*; Aminoff & Sears, 1971) and in particular for their release by spinal cord section (Downman, 1955; Downmann & Hussain, 1958). The upper lesions we used include the dorsolateral quandrant which Downman & Hussain (1958) found to be critical in the release of splanchnic to intercostal and intercostal to intercostal reflexes.

Cross-correlation histograms with central peaks, such as those reported here, can be generated either by common excitatory inputs or by common inhibitory ones (Moore, Segundo, Perkel & Levitan, 1970). The lesions used by Downman & Hussain (1958) released both excitatory and inhibitory effects. The Renshaw cells of the thoracic cord, whose inhibitory effects may also be potentiated by the lesions used here (Kirkwood *et al.* 1981*b*) are thus candidate neurones for the generation of the broad-peak synchronization, at least in the lesioned animals.

The release of thoracic interneurones by lower thoracic lesions has not been

described before. Indeed, Downman (1955) reported no change in intercostal to intercostal reflexes above a mid-thoracic (T8) transection, although his chances of seeing a change were minimized by his use of a maximal stimulus. In unpublished work T. A. Sears found, by the use of a near threshold test stimulus, that a low thoracic/upper lumbar transection caused a considerable potentiation of a similar intercostal reflex, as might be expected by analogy with the Schiff–Sherrington phenomena (Ruch & Watts, 1934). However, this analogy cannot be carried very far. In the Schiff–Sherrington phenomenon, the forelimb reflexes above a low thoracic transection show enhanced extensor tone. The broad-peak synchronization here has more of the properties of a flexor effect in that it was absent in three decerebrate animals and was potentiated by an upper lesion, as are flexor reflexes (Eccles & Lundberg, 1959; Kuno & Pearl, 1960). However, an alternative possibility is that the interneurones released form a heterogenous group and thus show some flexor-like and some extensor-like properties.

Identifying the mechanism that synchronizes the interneurones is a separate question from identifying the neurones. There are at least three possibilities, all of which may operate together. Firstly, the branched axon mechanism which produces short-term synchronization of the motoneurones is a general one and as such will exert its effects on pools of interneurones. If it is sufficiently strong and if there are many interconnexions, then the multi-synaptic chains involved could produce a gradual broadening of the narrow peaks which are appropriate to a monosynaptic common input system. Secondly, the interneurones concerned could have patterned discharges, e.g. they could fire in bursts. Thirdly an external synchronizing stimulus, such as the cardiac pulse which can synchronize intercostal muscle spindles (Kirkwood & Sears, 1982*a*), could be involved. The observation of the broad peaks with the dorsal roots cut is not a good control against this third possibility because, with polysynaptic pathways, the synchronization could be transmitted over several segments or from the contralateral cord (Kirkwood *et al.* 1982).

The pathways which control the interneurones are unknown. The interneurones may be part of a descending polysynaptic pathway transmitting the respiratory drive in parallel with the monosynaptic pathway so as to allow for integration with segmental and other inputs (Aminoff & Sears, 1971) and thus be excited by the respiratory drive. Alternatively they may be inhibited by the respiratory drive. If this latter possibility were true and the interneurones represented inputs from other systems, then there would be teleological appeal if, at high CO_2 when a strong respiratory effort is required, the respiratory drive itself were responsible for shutting down the pathways from the less essential inputs.

At a finer level of control, it is likely that synchronization, particularly of the strong broad-peak type, is undesirable behaviour for motoneurones. It must contribute to tremor (cf. Dietz *et al.* 1976), despite this not being so in the specialized conditions of the tonic vibration reflex (Clarke, Matthews & Muir, 1981). The effects of the lesions then may be seen as the removal of some of the controls which normally limit the spread of this synchronization (cf. Rudomín, Burke, Núñez, Madrid & Dutton, 1975). The effects of such lesions are even more pronounced when time is allowed for functional and structural re-organization of the synaptic inputs (Kirkwood *et al.* 1981*a* and in preparation), a process which has a long time scale anatomically (Pullen

& Sears, 1978). Other procedures can also open up these synchronized pathways, such as strength training (Milner-Brown, Stein & Lee, 1975) where the extra force may be presumed to be gained at the expense of the fine control which may be diminished by the synchronization. The existence of the pathways involved in such synchronization, however, is to be expected in a normal animal, since synchronized behaviour may be required (in the case of the intercostals) for such tasks as vocalization (cf. Sears & Stagg, 1976) or the defensive reflexes of the respiratory tract (Tomori & Widdicombe, 1969). Thus although the strongest broad-peak synchronization which we are reporting here is an abnormal feature resulting from acute lesions it may well represent the time scale or mode of activation of pathways contributing to normal intercostal movements, which are part of a spectrum ranging from ballistic movements, via respiratory to those which contribute to posture as a whole.

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